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# Biochemical and Molecular Epidemiology of Human Cancer: Indicators of Carcinogen Exposure, DNA Damage, and Genetic Predisposition

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**The primary goal of biochemical and molecular epidemiology is to identify individuals at high cancer risk by obtaining evidence of high exposure to carcinogens, leading to pathobiological lesions in target cells, and/or increased oncogenic susceptibility due to either inherited or acquired host factors. This emerging and multidisciplinary area of cancer research combines epidemiological and laboratory approaches. Because DNA is considered to be an important target for modification by mutagens and carcinogens, damage to DNA can be used as an internal, molecular dosimeter of carcinogen exposure. The reactive species of these carcinogens may directly bind to DNA to form adducts and may indirectly cause secondary DNA lesions, e.g., via induction of free radicals and aldehydes. Highly sensitive and specific methods have been developed to measure the minute amounts of DNA lesions and DNA repair products found in biological specimens from humans exposed to carcinogens. For example, DNA adducts have been measured in cells and tissues from people occupationally exposed to carcinogenic polycyclic aromatic hydrocarbons. Antibodies recognizing carcinogen-DNA adducts have also been detected in human sera. Inherited predisposition to cancer has been revealed by recent advances in molecular genetics, including restriction-fragment-length polymorphism. For example, the hypothesis that rare alleles of the Ha-*ras* proto-oncogene are associated with an increased risk of lung cancer is currently being tested. These approaches afford the potential of biochemical and molecular epidemiology to predict disease risk for individual persons, instead of for populations, and before the onset of clinically evident disease.**

## Introduction

The primary goal of biochemical and molecular epidemiology is to identify individuals at high cancer risk by obtaining evidence of high exposure to carcinogens, leading pathobiological lesions in target cells and/or increased oncogenic susceptibility due to either inherited or acquired host factors. This emerging and multidisciplinary area of cancer research combines epidemiological and laboratory approaches (*1–3*). Clinical and epidemiological studies have identified populations at high cancer risk and, in many cases, also the etiological agents, e.g., tobacco smoke as the major cause of bronchogenic carcinoma. Laboratory studies have extended these epidemiological findings by isolating and identifying individual carcinogenic and cocarcinogenic agents found in complex mixtures, e.g., polycyclic aromatic hydrocarbons (PAH) in coal tar, diesel exhaust, and tobacco smoke, and by discovering naturally occurring and man-made carcinogenic and cocarcinogenic agents in our environment. Laboratory studies, especially those using animal models, have also made major contributions to our current understanding of the multistage carcinogenic process that has been suggested by clinical observations, human histopathologic, and investigations of animal models. Extrapolation of carcinogenesis data to humans and the interindividual variation in oncogenic susceptibility in the human population have been previously discussed in detail (*4,5*).

This report will discuss opportunities and some of the experimental approaches that show promise in identifying individuals at the highest risk in a population of people at high cancer risk. The concepts of tumor initiation, promotion, malignant conversion, and progression developed from studies in chemical carcinogenesis (*6–9*) form the framework for this discussion. A schematic representation of this multistage process is depicted in Figure 1. Humans are repeatedly exposed to complex and variable mixtures that contain both initiators and promoters, e.g., tobacco smoke (*10*), and to complete carcinogens having both initiating and promoting activities. Other factors, including host factors,

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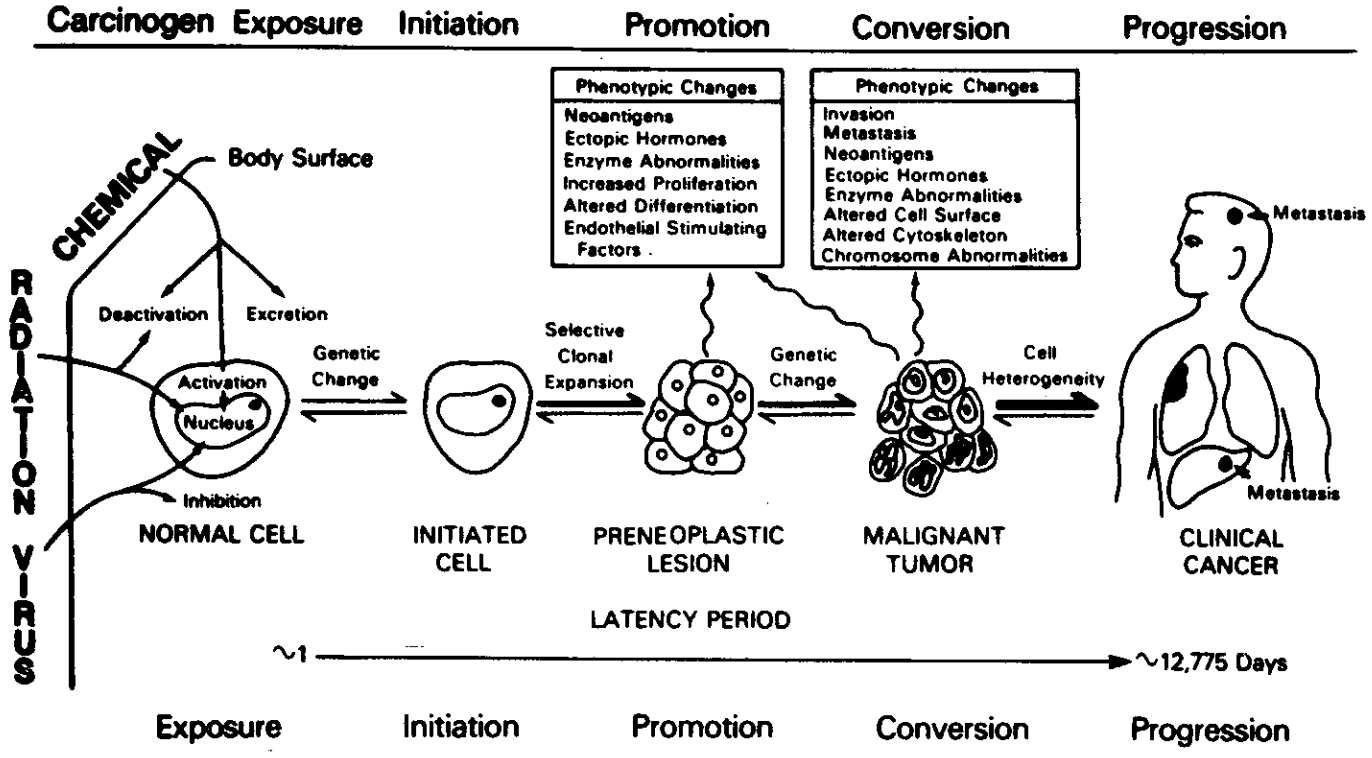


FIGURE 1. Schematic representation of the multistage process of carcinogenesis. Examples of factors that may either enhance or inhibit carcinogenesis are taken from studies of experimental carcinogenesis.

are also known to influence cancer risk (*4,5,11–13*). This concept of multistage carcinogenesis provides a bridge between experimental animal studies, *in vitro* human cell carcinogenesis, and human carcinogenesis (*14*).

The earliest events in the multistage process of chemical carcinogenesis are considered to include exposure to carcinogens, transport of the carcinogen to the target cell, activation to ultimate carcinogenic metabolites, if the agents are procarcinogens, and DNA damage leading to changes that result in the initiated cell (Fig. 1). Each of these events has been extensively reviewed (*2,9*) and will only be briefly discussed here.

## Carcinogen Exposure and Metabolism

Evidence of human exposure to carcinogens can be indirectly obtained by detection of carcinogens in the environment. Direct evidence comes from measurement of carcinogens and/or their metabolites in body fluids (*15*), such as urine (*16–21*), breast milk (*22,23*), seminal fluid (*24*), and serum (*15,25–27*), and in samples of tissue that may accumulate the carcinogen, such as fat for lipophilic agents (*28–30*). Mutagenic activity found in body fluids or extracts of tissues has also been used as an indirect measurement of the putative presence of carcinogens (*22,31–33*).

Once the carcinogen is transported into the target cell, it may be enzymatically activated, generally to an electrophilic metabolite (*34*) and may be detoxified, generally to more polar, water-soluble metabolites. Many carcinogens in our environment are procarcinogens that require enzymatic activation. It has been recognized during the last decade that many carcinogens require multiple enzymatic steps to become activated to their ultimate carcinogenic form, and the activity of the rate-limiting enzymatic reaction responsible for activation of a carcinogen may differ among people and influence their cancer risk. Therefore, the metabolic balance between activation and deactivation of a procarcinogen and the covalent binding of its ultimate carcinogenic metabolite to DNA are considered to be important events in stages of carcinogenesis tumor termed initiation and malignant conversion (Fig. 2).

Carcinogens can also be formed by endogenous reactions, e.g., nitrosation of amines to form *N*-nitrosamines. Assays to assess an individual's capability of producing endogenous carcinogens are being developed using safe substrates. For example, nitrosation of proline to form *N*-nitrosoproline, which is excreted in urine, can be readily measured (*35*). Certain studies suggest that endogenous nitrosation may be increased in tobacco smokers (*36,37*), whereas another study did not confirm these results (*38*).

Most chemical carcinogens are activated by oxidative metabolism. The increasing number of types of cytochrome P-450 that have been identified in laboratory

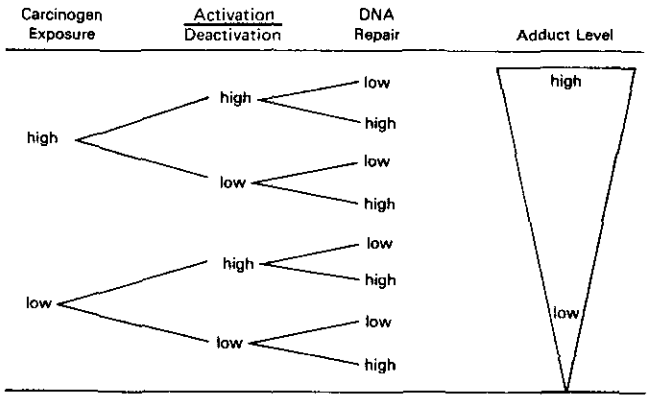


FIGURE 2. Major determinants of amounts of carcinogen-DNA adducts.

animals (*39*) and in human tissues (*40–42*) may in part explain interindividual differences in xenobiotic metabolism; interindividual variations in the activities of enzymes responsible for carcinogen metabolism in various human tissues and cells vary more than 1000-fold (*43,44*). Substrate affinities and/or capacities of these multiple forms differing from person to person is another possible important variable factor.

Certain carcinogens and drugs may be metabolically activated by the same enzymes, and indicator drugs, such as debrisoquine, antipyrine, and dapsone, may prove to be useful in probing genetic polymorphisms of carcinogen metabolism (*43,45*). Using debrisoquine metabolic ratio (debrisoquine/4-hydroxydebrisoquine in the urine), Idle and co-workers (*46*) have found an increased rate of hydroxylation of debrisoquine in patients with either bronchial cancer or hepatocellular cancer as compared to that of noncancer patients. In bronchial cancer patients, this increase was found to be the case irrespective of the number of cigarettes consumed.

*N*-Acetylation polymorphism is the best-known genetic condition affecting the pharmacokinetics of several drugs, e.g., isoniazid, hydralazine, procainamide, and dapsone (*47*). The acetylation status is controlled by two autosomal alleles at a single locus, with slow acetylation being manifest as a recessive trait. There is some evidence that an increased urinary bladder cancer risk in those occupationally exposed to carcinogenic aromatic amines might be due to their slow *N*-acetylation phenotype (*48,49*). In contrast, the fast *N*-acetylation phenotype is associated with increased risk of colon carcinoma (*50*).

## Carcinogen-Macromolecular Adducts

As noted in the previous section, metabolism of carcinogens from several chemical classes, including *N*-nitrosamines, polycyclic aromatic hydrocarbons, hydrazines, mycotoxins, and aromatic amines, have been in human tissues and cells (*51–54*). The enzymes responsible for the activation and deactivation of procarcinogens, the metabolites produced, and the carcinogen-DNA adducts formed by cultured human tissues and cells are generally qualitatively similar among donors and tissue types. The DNA adducts and carcinogen metabolites are also very similar to those found in most laboratory animals, an observation that supports the qualitative extrapolation of carcinogenesis data from the laboratory animal to the human situation. Because studies using experimental animals generally indicate that their cancer risk is influenced by the capacity for metabolic activation of procarcinogens, it is likely that a similar relationship exists for humans (*55,56*).

Table 1 lists examples of procarcinogens activated by cultured human tissues into metabolites that bind covalently to DNA. Although the major DNA adducts are qualitatively similar, quantitative differences have been found among individuals and their various tissues, and in outbred animals. These differences in enzymatic activities and number of DNA adducts generally range from 10- to 150-fold among humans and are of the same order of magnitude found in pharmacogenetic studies of drug metabolism.

Results from *in vivo* and *in vitro* studies discussed above serve as a basis for investigations in biochemical and molecular epidemiology. For example, the observation that the carcinogen-DNA adducts formed in cultured human tissues are generally the same as those found in experimental animals in which these chemicals induce cancer has encouraged investigators to search for DNA adducts in biological specimens obtained from people exposed to either specific carcinogens such as benzo[a]pyrene or chemotherapeutic agents. The recent development of highly sensitive methods for detecting carcinogen-DNA adducts has made this search possible (Table 2). Methods currently used include $^{32}$P-nucleotide postlabeling and chromatography (*57*), synchronous scanning fluorescent spectrophotometry (*58,59*), mass spectrometry (*60,61*), and immunoassays (*62–65*). These methods are being used to measure carcinogen-macro-

**Table 1. Interindividual variation in carcinogen binding to DNA in cultured human tissues.[a]**

| Tissue | Fold variation in carcinogen binding | | | | | |
|---|---|---|---|---|---|---|
| | BP | $AFB_1$ | DMNA | 1,2-DMH | AAF | DMBA |
| Esophagus | 99 | 70 | 90 | | | |
| Trachea | 6 | | | | | |
| Bronchus | 75 | 120 | 60 | 10 | 18 | 50 |
| Peripheral lung | 3 | | | | | |
| Liver | | 12 | | | | |
| Duodenum | 31 | | | | | |
| Colon | 197 | 150 | 145 | 80 | | |
| Bladder | 68 | 127 | | | 14 | |
| Endometrium | 70 | | | | | |

[a] The highest variation among people was reported for benzo[a]pyrene (BP), aflatoxin $B_1$ ($AFB_1$), dimethylnitrosamine (DMNA), 1,2-dimethylhydrazine (1,2-DMN), acetylaminofluorene (AAF), and 7,12-dimethylbenzanthracene (DMBA), carcinogen-DNA binding in cultured human tissues (*14,44*). The number of cases studied ranges from less than 10, e.g., peripheral lung, to more than 100, e.g., colon in the various investigations.

**Table 2. Examples of assays to measure carcinogen-marcromolecular adducts.**

| |
|---|
| Cellular DNA adducts |
| Nonhydrolyzed |
| Immunoassays |
| Fluorimetry |
| Other |
| Hydrolyzed (concentration, e.g., HPLC or immunocolumn, followed by): |
| $^{32}$P-Postlabeling |
| Immunoassay |
| Fluorimetry |
| Other |
| Urinary base adducts |
| Protein adducts |
| Hemoglobin |
| Albumin |

molecular adducts in cells from people exposed to carcinogenic chemicals (Tables 3–6) and cancer chemotherapeutic agents (*66*). Although these techniques measure DNA lesions considered to be important in carcinogenesis, this is a multistage process, so it is unlikely that DNA adducts will be precise quantitative predictors of cancer risk.

Our research group has primarily used two complementary approaches for measuring PAH-DNA adducts: ultrasensitive enzyme radioimmunoassay (USERIA) and synchronous scanning fluorescent spectrophotometry (SFS). These assays have been validated using animal models and cells *in vitro* (*67,68*). The aim of our initial epidemiological studies has been to determine if PAH-DNA adducts and serum antibodies recognizing these adducts can be detected in biological samples from people exposed to high levels of PAH, e.g., coke oven workers and roofers.

Among a group of 27 U.S. coke-oven workers who had been employed for between 5 and 30 years, 18 (67%) were found to be carrying significant levels of adducts in their peripheral blood lymphocytes (*69*) (Table 3). Within this group, 12 were smokers and 15 were nonsmokers; of the smokers, 9 (75%) were positive, and of the nonsmokers, 9 (60%) were positive. Seven members of this group were known to work in environments containing high levels of benzene-soluble particulates, and 6 of them (*86%*) were found to be positive in the USERIA test. In a study involving 3 groups, i.e. 28 roofers, 20 foundry workers, and 9 laboratory workers, 7 (25 and 35%) of the samples from each of the groups subjected to PAH exposure and 2 (22%) of the nonindustrially exposed individuals were found to be positive by USERIA (*70*). Within a group of 38 Norwegian coke-over workers, 13 (34%) individuals were found to have detectable, peripheral blood lymphocyte DNA adducts (*70*); among these, 6 were smokers. Following a 3-week vacation period, 9 out of the group of 13 had lower but still detectable levels of adducts in the USERIA assay.

These assays employed a rabbit antiserum developed by immunization with benzo[a]pyrene diol epoxide (BPDE)-DNA (*71*). The specificity of the rabbit antiserum used in these tests was determined by the examination of the extent to which its reactivity to BPDE-DNA was inhibited by the related adducts formed when benz[a]anthracene diol epoxide (BADE) and chrysene diol epoxide (ChDE) were reacted with DNA *in vitro*. The inhibition curves show that both BADE-DNA and ChDE-DNA cross-react with the anti-BPDE-DNA antiserum to a significant extent, but the antiserum does not react with carcinogens of other chemical classes, e.g., aromatic amines and mycotoxins (*67,68,71*). Therefore, the rabbit antiserum recognizes certain other

**Table 3. Detection of DNA adducts in human tissues and cells by enzyme immunoassays.**

| Immunoassay | Origin of DNA | No. positive/ No. tested, % | Approximate level of adducts per $10^8$ nucleotides | Reference |
|---|---|---|---|---|
| BPDE-DNA antigenicity | Placenta | | | (*102*); Santella et al., unpublished data |
| | Smokers | 23/25 (92) | 60 | |
| | Nonsmokers | 22/25 (88) | 20 | |
| | Lung | | | |
| | Lung cancer patients | 5/16 (31) | | |
| | COPD | 0/1 (0) | 5–10 | (*103*) |
| | Control or other cancer | 0/10 (0) | | |
| | Coke oven workers | | | |
| | Finland | 7/20 (35) | 10 | (*20*) |
| | Norway | 13/36 (36) | 3–490 | |
| | USA | 18/27 (67) | 75–420 | (*69*) |
| | Roofers | 7/28 (25) | 20 | (*70*) |
| | Foundry workers | 7/20 (35) | 20 | (*70*) |
| $O^6$-methylguanine | Chinese cancer patients: esophageal and stomach mucosa | 27/37 (73) | <1–10 | (*104*) |
| | European cancer patients: esophageal and stomach mucosa | 7/14 (50) | <2 | (*104*) |
| $O^6$-ethylguanine | Chinese and European cancer patients | 0/51 (0) | — | (*104*) |

**Table 4. Detection of DNA adducts in human tissues and cells by fluorescent assay.**

| Adduct | Origin of DNA | No. positive/ No. tested, % | Approximate level of adducts per $10^8$ nucleotides | Reference |
|---|---|---|---|---|
| PAH-DNA[a] | Lymphocytes | | | |
| | Coke oven workers | | | |
| | USA | 31/41 (76) | 10–100 | (*69*) |
| | Norway | 4/13 (31) | 15–80 | (*59*) |
| 7-Methylguanine | Liver | | | |
| | DMN poisoning | 2/2 (100) | $5 \times 10^5$ | (*105*) |
| | MeBr poisoning | 0/1 (0) | — | |
| | Reye's syndrome | 0/3 (0) | — | |
| | Kidney | | | |
| | MeBr poisoning | 0/1 (0) | — | |
| $O^6$-methylguanine | Liver | | | |
| | DMN poisoning | 2/2 (100) | $1 \times 10^5$ | (*105*) |
| | MeBr poisoning | 0/1 (0) | — | |
| | Reye's syndrome | 0/3 (0) | — | |
| | Kidney | | | |
| | MeBr poisoning | 0/1 (0) | — | |

[a] Spectra similar to benzo[a]pyrene tetrol.

**Table 5. Detection of putative DNA adducts in human tissue and cells by $^{32}$P-postlabeling analysis.**

| Adduct | Origin of DNA | No. positive/ No. tested, % | Approximate level of adducts per $10^8$ nucleotides | Reference |
|---|---|---|---|---|
| Unknown DNA (CSC-related) | Placenta | | | |
| | Smokers | 29/30 (97) | < 0.1–1.4 | (*102,106*) |
| | Nonsmokers | 3/24 (12) | | |
| Unknown DNA | Oral mucosa | | | |
| | Betel nut chewers | 22/22 (100) | < 0.1–10 | (*107*) |
| | Tobacco chewers | 22/22 (100) | | |
| | Inverted smokers | 15/15 (100) | | |
| | Ethnic controls | 19/19 (100) | | |
| | Bone marrow cells | | | |
| | Smokers | 4/4 (100) | < 0.1–9 | (*108*) |
| | Nonsmokers | 6/6 (100) | | |
| | Bronchus | | | |
| | Smokers | 1/1 (100) | < 17–29 | (*106*) |
| | Nonsmokers | 0/1 (100) | | |

**Table 6. Detection of protein adducts in human tissues and cells by physical assays.**

| Adduct | Origin of hemoglobin (hb) | No. positive/ No. tested, % | Approximate level detected, pmole/g | Reference |
|---|---|---|---|---|
| Aminobiphe nyl-hb | Smokers | 30/30 (100) | 0.37–1.5 | (*109*) |
| | Nonsmokers | 38/38 (100) | 0.04–0.64 | |
| Ethylene-oxide (ETO)valine | Smokers | 11/11 (100) | 217–690 | (*61*) |
| | Nonsmokers | 14/14 (100) | 27–106 | |
| Ethylene-oxide valine | EtO workers | 7/7 (100) | 20–7700 | (*60*) |
| | Controls | 3/3 (100) | 30–930 | |
| Ethylene-oxide histidine | EtO workers | 7/7 (100) | 550–8000 | (*60*) |
| | Controls | 3/3 (100) | 530–1600 | |

PAH-DNA adducts and provides a wide screen for detecting DNA modified by certain PAHs.

Fluorimetric assays have also been useful in detecting PAH-DNA adducts (Table 4). For example, DNA samples isolated from the peripheral blood lymphocytes of 41 U.S. coke-oven workers were examined by SFS using a fixed wavelength interval of 34 nm to simultaneously drive the excitation and emission monochromators (*69*). Fluorescent emissions that were similar to the signals generated for either authentic BPDE-DNA adducts or BPDE-DNA adducts isolated from mouse skin were detected in 31 cases (75%). However, two types of complex spectra were detected: sharp peaks (24%) and broad peaks (51%) (Fig. 3). These data suggest the presence of more than one fluorophore in these samples that contribute to the signal and therefore do not allow for precise quantitation without further separation methodology, as will be discussed below. However, presence of the sharper type of peaks with fluorescent emission maxima in the region expected for pyrene (374–384 nm) suggest the presence in these samples of BPDA-DNA adducts. When DNA samples from 38 Norwegian coke-oven workers were examined by SFS, only 4 (10%) were found to have fluorescent emissions between 374 and 384 nm (*20*). These corresponded to 4 of the 13 samples that were positive in the USERIA, and 3 (75%) were smokers. Following a vacation period (3 weeks), however, no fluorimetric evidence for the presence of BPDE-DNA adducts was found in this group of coke-oven workers, and substantial decreases were also found by USERIA.

The use of a physical assay (SFS) in these studies complements the immunoassays, and it appears that each of the assays demonstrate the formation of a range of PAH-DNA adducts in many of the individuals studied. Data obtained in any individual assay are clearly less reliable than if those data are substantiated by duplication in an alternative system. Positive results obtained by both SFS and USERIA strongly suggest the presence of the activated BP or closely related moiety in a given DNA sample. Two types of fluorescent signal, a broad type and a sharp type, are detected by SFS. The sharp type is probably indicative of a relatively large concentration of BPDA-DNA adducts and this assertion is substantiated by comparison of the SFS and USERIA, where data for both assays are available. In addition, it has been possible to fractionate lymphocyte-DNA samples by high pressure liquid chromatography for SFS and in some cases clearly define the identity of the fluorescent signals (*68*).

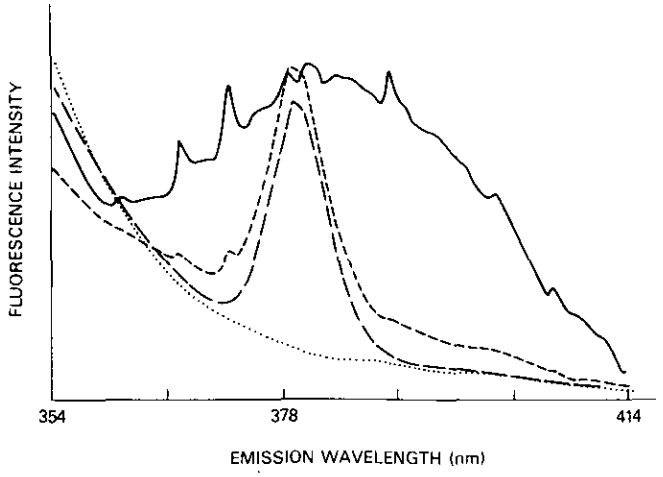


FIGURE 3. Synchronous fluorescence spectra of synthetic BPDE-DNA (···) adducts and DNA samples from peripheral blood lymphocytes of coke-oven workers. The broad peak (—) was found to be carrying adducts corresponding to 1.2 fmole BPDE/μg DNA by USERIA and the sharp peak (---) 26.5 fmole BPDE/μg DNA.

The $^{32}$P-postlabeling method developed by Randerath and co-workers (*57*) enables the fingerprinting of modified deoxynucleotides isolated from tisses of experimental animals and humans exposed to complex mixtures of environmental carcinogens. This is proving to be a highly sensitive screening method because detection of both previously characterized DNA adducts and unknown adducts is possible (Table 5). In fact, more than nine unidentified putative DNA adducts have been detected by this method in bone marrow, placenta, and lung tissue from cigarette smokers or nonsmokers. The chemical identification of these unknown spots on the chromatograms and the precise quantitation awaits further study.

Although hemoglobin and albumin are not considered targets for the pathobiological effects of carcinogens, these macromolecules have certain advantages for the molecular dosimetry of exposure to carcinogens. For example, red blood cells have a lifetime of about 120 days in humans, so the levels of carcinogen-hemoglobin adducts may be an integrative measure of exposure during a period of nearly 4 months. In addition, these protein adducts, unlike DNA adducts, are not considered to be repaired in the circulating red blood cells, and milligram quantities can be obtained from a few milliliters of blood. Ehrenberg and co-workers (*72*) pioneered this area of research for alkylating agents such as ethylene oxide. The amounts of ethylene oxide and 4-aminobiphenyl-hemoglobin adducts are substantially increased in tobacco smokers compared to nonsmokers (Table 6).

Interestingly, antibodies directed against authentic BPDE-DNA adducts have been found in human sera (*69*). Antibody binding patterns were established for these samples, and it was found that 11 (27%) of the individuals in one study had developed antibodies to BPDE-DNA adducts. In a separate study (*20*) where serum was obtained from 38 donors at two sampling times (9 months apart), 12 (32%) and 13 (34%) serum samples, respectively, were found to contain antibodies that were directed against synthetic BPDE-DNA adducts (*20*).

Analyses of human antisera by enzyme immunoassays showed that antibodies are produced against a range of PAH-DNA adducts (Newman et al., unpublished results). Selected sera were found to contain antibodies specific for BPDA-DNA, ChDE-DNA, and BADE-

DNA adducts, suggesting that these adducts each possess at least one immunologically unique epitope. In addition, the results of antigen-competitive enzyme immunoassays showed the presence of both adduct-specific and cross-reactive antibodies in some sera. Cross-reactivity was documented between BADE-DNA and ChDE-DNA adducts and between ChDE-DNA and BPDE-DNA adducts, but not between BADE-DNA and BPDE-DNA adducts. Thus, the presence of two different cross-reactive epitopes is suggested, one on BPDE-DNA and one on BADE-DNA, both of which are shared with ChDE-DNA. Computer simulations of these adducts using methods described by Brooks (*73*) are being compared in an attempt to more fully understand the structural variations between these compounds that amount for the observations described.

Current dogma has directed our efforts to measuring adducts formed by the direct interaction between the activated carcinogen metabolite(s). However, carcinogens may also exert their oncogenic effects via indirect damage to macromolecules such as carcinogen-induced formation of superoxides, which cause DNA damage including thymine glycol and other altered nucleic acid structures. This induction of the pro-oxidant state, i.e., increased concentrations of active oxygen, organic peroxides, and radicals, may also be of importance in tumor promotion (*74*). Both immunoassays (*63,75*) and $^{32}$P-postlabeling assays (*76*) are being developed to measure radiation- and radical-induced DNA damage.

## Genetic Predisposition to Cancer

Due to the biologically diverse, genetically heterogeneous, human population and the multistep nature of the carcinogenic process, there are differences in susceptibility between individuals to the oncogenic effects of environmental carcinogens.

Investigation of individuals with multiple tumors has led to the discovery of several hereditary conditions of increased cancer incidence (Table 7) (*77,78*). These diseases may be transmitted by single-gene, polygenic, or chromosomal mechanisms. Some hereditary conditions predispose only one type of tissue of neoplasia (e.g., actinic keratosis predisposes to basal cell carcinomas of the skin and familial polyposis coli predisposes to tumors in the gastrointestinal tract), while other hereditary conditions predispose to multiple tumors in different tissues (e.g., hereditary retinoblastoma, Bloom's syndrome, and ataxia telangiectasia).

Most known hereditary causes of multiple tumors were discovered because they manifested as dramatic clinical syndromes with a Mendelian pattern of inheritance. In some of these diseases, host susceptibility to specific environmental agents has been determined, e.g., ultraviolet radiation as a cause of skin cancer in patients with xeroderma pigmentosum. In contrast to these rather rare conditions, most common types of hereditary multiple cancer occur in familial patterns which probably have a polygenic basis (*79*).

Analysis of certain epidemiological data has suggested a two-hit mutation model of carcinogenesis (*80*). A genetic predisposition may be due to inheritance of the first autosomal mutation so that only one additional genetic event is required. Therefore, these individuals may already have initiated cells due to germline genetic lesions. Retinoblastoma is an example of a human tumor in which a pair of tumor suppressor genes located at the chromosomal region 13q14 and loss (inactivation) of these diploid genes leads to tumor formation (*81*). In this context, diploidy can be viewed as a protective genetic mechanism. Possible mechanisms producing homozygosity or hemizygosity at the retinoblastoma locus in tumor cells include chromosomal nondisjunction, nondisjunction and reduplication, mitotic recombination, deletion, gene inactivation, and mutation. One of these mechanisms, mitotic recombination, may also be important in the genetic predisposition to cancer found in Bloom's syndrome.

Evidence for these presumed dominant-acting "tumor suppressor" genes has arisen primarily from epidemiological studies discussed above; molecular analysis of polymorphic DNA restriction fragments showing a reduction to homozygosity of chromosome 13 found in retinoblastoma and osteosarcoma (*82*), of chromosome 11 in Wilm's tumor (*83–86*) and bladder cancer (*87*), and of chromosome 22 in acoustic neuroma (*88*); and studies with human cell hybrids in the field of somatic cell genetics (*89–91*). Recently, a DNA with properties of the *Rb* gene, whose loss correlates with the development of retinoblastoma and osteosarcoma, has been isolated (*92*). Stanbridge and co-workers (*93*) have also reported

**Table 7. Examples of inherited disorders associated with an increased risk of cancer (*77*).**

| |
|---|
| Constitutional chromosomal abnormalities |
| Down's syndrome |
| Klinefelter's syndrome |
| Mendelian traits |
| Inherited cancer syndromes |
| Retinoblastoma |
| Familial polyposis coli |
| Multiple endocrine neoplasia syndromes |
| Inherited preneoplastic states |
| DNA repair defects and chromosomal instability syndromes: Xeroderma pigmentosum, Fanconi, Bloom's, ataxia telangiectasia |
| Disturbances of tissue organization |
| Hamartomatous syndromes: Peutz-Jegher's, Cowden, neurofibromatosis |
| Other conditions in which disturbance of tissue proliferation, differentiation or organization is associated with increased cancer risk: disorders of skin keratinization (?), α-1-antitrypsin deficiency (?) |
| Immune deficiency syndromes |
| Metabolic variation: |
| Albinism |
| Aryl hydrocarbon hydroxylase inducibility (?) |
| Variations in estrogen metabolism (?) |
| Multifactorial predisposition |
| Ethnic cancer differences |
| Familial cancer aggregations |

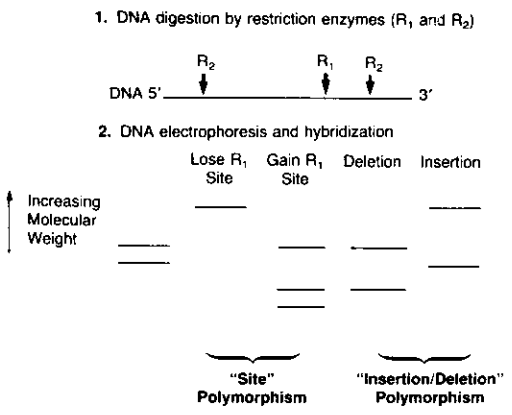


FIGURE 4. Schematic representation of DNA polymorphism analyzed by restriction enzyme digestion, DNA electrophoresis, and DNA hybridization.

suppression of tumorigenicity with continued expression of the C-Ha-*ras* oncogene in EJ bladder carcinoma-human fibroblast hybrid cells. This finding suggests that, even in the presence of an activated proto-oncogene, tumor suppressor genes are dominant-acting.

New experimental approaches to identify people genetically predisposed to cancer are being evaluated, e.g., restriction enzyme DNA fragment-length analysis of genetic polymorphism (RFLP). Until recently, genetic predisposition could be assessed only by measuring gene products, e.g., histocompatibility antigens on cell surfaces, isoenzymes and, as discussed earlier, pharmacogenetic phenotype. Advances in molecular biology now have made it possible to measure genetic polymorphism at the DNA level, which greatly expands the potential of assays of polymorphisms in biomedical research. Genetic polymorphism can be measured by restriction enzyme analysis and DNA hybridization (Fig. 4) (*94*). RFLPs are of two types: site or insertion/deletion polymorphism. In the case of site polymorphism, the recognition site for a given restriction enzyme in a particular region of DNA either appears or disappears as the result of point mutation. Accordingly (Fig. 4), in the site-absent phenotype, one large fragment instead of two smaller fragments is observed. Conversely, in the site-present phenotype, three fragments (one of expected size and two smaller) instead of two fragments are seen.

In the case of insertion/deletion polymorphism, variation in fragment length is the result of insertion or deletion of DNA sequences and will be detected by any restriction endonuclease that possesses recognition sites tightly spanning the region of sequence alteration. Because the insertions or deletions can assume a continuum of lengths, more than two alleles are possible. This RFLP approach has already been proven beneficial in identifying individuals with a genetic predisposition to a variety of diseases, including retinoblastoma, non-insulin-dependent diabetes mellitus, Huntington's disease, and hemoglobinopathies (*95*). The same molecular approach using specific DNA probes, e.g., for oncogenes and ectopic hormones, is now being studied to determine its potential in identifying persons with a genetic predisposition to cancer. The human Ha-*ras* proto-oncogene may prove useful for these studies because it contains a hypervariable insertion/deletion polymorphism. The molecular basis for this insertion/deletion polymorphism is a region of 30 to 100 tandem nucleotide repeats with a 28 base pair consensus sequence aligned head-to-tail that is approximately 1 kilobase downstream from the structural gene of Ha-*ras*. The function of this variable tandem repeat (VTR) is unknown but it may have gene enhancer activity and appears to be inherited in a Mendelian fashion. An increased frequency of rare alleles at this locus has been found in certain cancer patients, including those with bladder carcinoma (*96*) and breast carcinoma (*97*). Preliminary data indicates that rare alleles are also more frequent in patients with bronchogenic carcinoma (Weston et al., unpublished results). Evidence for (*98*) and against (*99*) Ha-*ras* involvement in sporadic and familial melanoma has been reported.

Although RFLP analysis should help to provide the general location of genes involved in common malignancies, in most cases more powerful tools will be required to precisely identify them. The recent development of pulsed-field electrophoresis, combined with innovative cloning techniques (*100,101*) should prove valuable in gene isolation.

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